

=> dup rem 13

PROCESSING COMPLETED FOR L3
L4 15 DUP REM L3 (8 DUPLICATES REMOVED)

=> d l4 ibib ab 1-15

L4 ANSWER 1 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:522010 CAPLUS
DOCUMENT NUMBER: 137:104771
TITLE: Transgenic yeast expressing phosphatases for increase the efficiency of producing prenyl alcohol
INVENTOR(S): Tokuhiro, Kenro; Muramoto, Nobuhiko; Yamada, Yukio; Asami, Osamu; Hirai, Masana; Ohto, Chikara; Obata, Shusei; Muramatsu, Masayoshi
PATENT ASSIGNEE(S): Kabushiki Kaisha Toyota Chuo Kenkyusho, Japan; Toyota Jidosha Kabushiki Kaisha
SOURCE: PCT Int. Appl., 93 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002053751	A1	20020711	WO 2001-JP11223	20011220
W: CA, CN, IN, JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
PRIORITY APPLN. INFO.:			JP 2000-401515	A 20001228
			JP 2000-401806	A 20001228

AB This invention provides a process of increasing prenyl alc. prodn. by transformation of phosphatases into yeast. The DNA and protein sequences of 6 phosphatase from different sources were disclosed. The expression of phosphate resulted in the activation of geranylgeranyl pyrophosphatase activity which assocd. with resulted the increase of the prodn. of prenyl alc.

REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:522004 CAPLUS
DOCUMENT NUMBER: 137:89441
TITLE: Repression of expression of squalene synthase in *Saccharomyces cerevisiae* to increase the efficiency of production of prenyl alcohol
INVENTOR(S): Ohto, Chikara; Obata, Shusei
PATENT ASSIGNEE(S): Toyota Jidosha Kabushiki Kaisha, Japan
SOURCE: PCT Int. Appl., 266 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002053747		20020711	WO 2001-JP11213	20011220
W: CA, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
JP 2002199883	A2	20020716	JP 2000-401701	20001228
PRIORITY APPLN. INFO.:			JP 2000-401701	A 20001228
			JP 2000-403067	A 20001228
			JP 2001-282978	A 20010918

AB This invention provides a process of repression of squalene synthase in *Saccharomyces cerevisiae* to increase the efficiency of prodn. of prenyl alc. The repression of squalene synthase expression was complemented by replacing the promoter of squalene synthase gene into GAL1 promoter. The isopentenyl diphosphate synthesis pathway assocd. enzymes, farnesyl diphosphate synthase, acetyl-CoA-acetyltransferase, hydroxymethylglutaryl CoA synthase, hydroxymethylglutaryl CoA reductase, mevalonate kinase, mevalonate phosphate kinase, isopentenyl diphosphate .DELTA.-isomerase from *Saccharomyces cerevisiae* were transformed into expression host. DNA sequences for farnesyl diphosphate synthase, geranylgeranyl diphosphate synthase and hydroxymethylglutaryl CoA reductase as well as the sequence of its mutated genes were provided. The invention also provides detailed description of expression vector construction for the enzymes expression in yeast.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE

FORMAT

L4 ANSWER 3 OF 15 CAPLUS COPYRIGHT 2002 ACS
 ACCESSION NUMBER: 2002:522002 CAPLUS
 DOCUMENT NUMBER: 137:90182
 TITLE: DNA and protein sequence of farnesyl diphosphate synthase and geranylgeranyl diphosphate synthase and their uses for producing prenyl alcohol
 INVENTOR(S): Ohto, Chikara; Obata, Shusei; Muramatsu, Masayoshi; Nishi, Kiyohiko; Totsuka, Kazuhiko
 PATENT ASSIGNEE(S): Toyota Jidosha Kabushiki Kaisha, Japan
 SOURCE: PCT Int. Appl., 337 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: Japanese
 FAMILY ACC. NUM. COUNT: 3
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002053746	A1	20020711	WO 2001-JP11214	20011220
W: CA, CN, IN, JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
PRIORITY APPLN. INFO.:			JP 2000-403067	A 20001228
AB This invention provides DNA and protein sequence farnesyl diphosphate synthase of <i>Saccharomyces cerevisiae</i> and geranylgeranyl diphosphate synthase of <i>E. coli</i> . The invention also provides the process of cloning of farnesyl diphosphate synthase, acetyl-CoA-acetyltransferase, hydroxymethylglutaryl CoA synthase, hydroxymethylglutaryl CoA reductase, mevalonate kinase, mevalonate phosphate kinase, isopentenyl diphosphate .DELTA.-isomerase from <i>Saccharomyces cerevisiae</i> . The invention also provides detailed description of expression vector construction for the enzymes expression in yeast and <i>E. coli</i> . The enzymes can be used for biosynthesis of prenyl alc. such as farnesol and nerolidol.				
REFERENCE COUNT:		7	THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE	
FORMAT				

L4 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:276135 CAPLUS
 DOCUMENT NUMBER: 136:291636
 TITLE: Improved ethanol production using
 thermophilic strains of **Bacillus**
 INVENTOR(S): Javed, Muhammad; Cusdin, Fiona; Milner, Paul; Green,
 Edward
 PATENT ASSIGNEE(S): Elsworth Biotechnology Limited, UK
 SOURCE: PCT Int. Appl., 44 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002029030	A2	20020411	WO 2001-GB4434	20011005
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2002081677	A1	20020627	US 2001-971361	20011005
PRIORITY APPLN. INFO.:			GB 2000-24554	A 20001006
			US 2000-247017P	P 20001113

AB The present invention relates to the prodn. of ethanol as a product of
 fermn of a thermol. **Bacillus** sp. In particular this invention
 relates to a novel method of gene inactivation and gene expression based
 upon homologous recombination. The invention shows that **ethanol**
prodn. may be improved through stabilization of a ldh (lactate
 dehydrogenase) gene mutation using transposon mutagenesis and homologous
 recombination in **Bacillus** strain TN. Furthermore, the PDC operon
 contg. pdc (pyruvate decarboxylase) gene from Zymomonas mobilis
 and adh (alc. dehydrogenase) gene from **Bacillus** strain LN may be
 expressed in the said strain for improved **ethanol prodn.**
 . The invention further claims the prodn. of ethanol using fermn. at a
 temp. between 40-75°C and a pH of 5.5-7.5. with air sparging in the
 culture such that the redox potential is between -360 and -400 mV.
 Furthermore, a process for continuous prodn. of ethanol in which the feed
 diln. rates are between 0.3-0.8 h-1 is provided. The inventors have
 produced sporulation deficient variants of a thermophilic, facultatively
 anaerobic, Gram-pos. bacterium which exhibit improved **ethanol**
prodn.-related characteristics.

L4 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 1
 ACCESSION NUMBER: 2002:209986 CAPLUS
 DOCUMENT NUMBER: 136:368511
 TITLE: Flux through citrate synthase limits the growth of
 ethanologenic *Escherichia coli* K011 during xylose
 fermentation
 AUTHOR(S): Underwood, S. A.; Buszko, M. L.; Shanmugam, K. T.,
 Ingram, L. O.
 CORPORATE SOURCE: Institute of Food and Agricultural Sciences,
 Department of Microbiology and Cell Science,
 University of Florida, Gainesville, FL, 32611, USA
 SOURCE: Applied and Environmental Microbiology (2002), 68(3),
 1071-1081
 CODEN: AEMIDF; ISSN: 0099-2240
 PUBLISHER: American Society for Microbiology
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB Previous studies have shown that high levels of complex nutrients (Luria

broth or 5% corn steep liquor) were necessary for rapid ethanol prodn. by the ethanologenic strain Escherichia coli KO11. Although this strain is prototrophic, cell d. and ethanol prodn. remained low in mineral salts media (10% xylose) unless complex nutrients were added. The basis for this nutrient requirement was

identified as a regulatory problem created by metabolic engineering of an ethanol pathway. Cells must partition pyruvate between competing needs for biosynthesis and regeneration of NAD+. Expression of low-Km

Zymomonas

mobile pdc (pyruvate decarboxylase) in KO11 reduced the flow of pyruvate carbon into native ferment pathways as desired, but it also restricted the flow of carbon skeletons into the 2-ketoglutarate arm of the tricarboxylic acid pathway (biosynthesis). In mineral salts medium contg. 1% corn steep liquor and 10% xylose, the detrimental effect of metabolic engineering was substantially reduced by addn. of pyruvate. A similar benefit was also obsd. when acetaldehyde, 2-ketoglutarate, or glutamate was added. In E. coli, citrate synthase links the cellular abundance of NADH to the supply of 2-ketoglutarate for glutamate biosynthesis. This enzyme is allosterically regulated and inhibited by high NADH concns. In addn., citrate synthase catalyzes the first committed step in 2-ketoglutarate synthesis. Oxidn. of NADH by added acetaldehyde (or pyruvate) would be expected to increase the activity of E. coli citrate synthase and direct more carbon into 2-ketoglutarate, and this may explain the stimulation of growth. This hypothesis was tested, in part, by cloning the **Bacillus subtilis** citZ gene encoding an NADH-insensitive citrate synthase. Expression of recombinant citZ in

KO11

was accompanied by increases in cell growth and ethanol prodn., which substantially reduced the need for complex nutrients.

REFERENCE COUNT: 55 THERE ARE 55 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE

FORMAT

L4 ANSWER 6 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:507865 CAPLUS
DOCUMENT NUMBER: 135:104937
TITLE: **Ethanol production by thermophilic strains of Bacillus sp.**
INVENTOR(S): Green, Edward; Baghaei-Yazdi, Namdar; Javed, Muhammad
PATENT ASSIGNEE(S): Elsworth Biotechnology Limited, UK
SOURCE: PCT Int. Appl., 30 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001049865	A1	20010712	WO 2001-GB36	20010105
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
US 2002034816	A1	20020321	US 2001-754083	20010105
PRIORITY APPLN. INFO.:			GB 2000-185	A 20000106
			US 2000-177199P	P 20000121

AB This invention relates to ethanol prodn. as a product

of bacterial fermn. In particular, the invention relates to ethanol prodn. by ~~thermophilic~~ strains of ~~Bacillus~~ sp. The invention describes the incorporation of heterologous gene pdc5 of *S. cerevisiae* or *Z. mobilis* into the chromosome of the gram-pos. bacterium. The bacterium is transformed with plasmid pFC1, more preferably with pFC1-PDC1. The invention further claims the prodn. of ethanol at a temp. between 40-750C.

L4 ANSWER 7 OF 15 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI
ACCESSION NUMBER: 1998-04018 BIOTECHDS

TITLE: Metabolic engineering of bacteria for ethanol production;

by transformation with the *Zymomonas mobilis* pyruvate-decarboxylase gene ; a review

AUTHOR: Ingram L O; Gomez P F; Lai X; Moniruzzaman M; Wood B E; Yomano L P; York S W

CORPORATE SOURCE: Univ. Florida-Inst. Food-Agr. Sci.

LOCATION: Department of Microbiology and Cell Science, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611, USA.

Email: lingram@micro.ifas.ufl.edu

SOURCE: Biotechnol.Bioeng.; (1998) 58, 2-3, 204-14

CODEN: BIBIAU

ISSN: 0006-3592

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The metabolic engineering of bacteria to convert lignocellulose into ethanol is reviewed. Topics include: lignocellulose is a challenging substrate for bioconversion; dilute hydrolysis of hemicellulose; enzymatic hydrolysis of cellulose; nutrients for lignocellulose-based fermentation; a hybrid approach for lignocellulose conversion to ethanol;

genetic engineering of bacteria to ferment hemicellulose sugars; improvements in ethanologenic *Escherichia coli*; fermentation of hemicellulose-derived sugars; genetic engineering of bacteria for cellulose fermentation; process optimization for cellulose fermentation;

ethanol production acid-treated bagasse;

ethanol production from office mixed waste-paper; other

improvements in the biomass conversion; fermentation of di-, tri-, and tetrasaccharides; and nutrients for the fermentation of lignocellulosic sugars. For **ethanol production**, the *Zymomonas*

mobilis pyruvate-decarboxylase (EC-4.1.1.1) gene has been expressed in *E. coli*, *Erwinia chrysanthemi*, *Klebsiella planticola*, *Klebsiella oxytoca*, *Enterobacter cloacae* and **Bacillus subtilis**.

(62 ref)

L4 ANSWER 8 OF 15 CEABA-VTB COPYRIGHT 2002 DECHEMA

ACCESSION NUMBER: 1997(06):4068 CEABA-VTB FILE SEGMENT B

DOCUMENT NUMBER: CEABA: 1997:1421890

TITLE: **Ethanol production** in gram-positive microbes

AUTHOR: Ingram, L. O N; Barbosa-Alleyne, M. D. F. (Univ. Florida, Gainesville, FL, USA)

SOURCE: US Patent (1996) US 5482846 (Appl. US 220072 Filed 30 Mar 1994)

CODEN: USXXAM

DOCUMENT TYPE: Patent

LANGUAGE: English

AB A gram-positive bacterium which was selected from **Bacillus subtilis** or **Bacillus polymyxa** is disclosed which was transformed with *Zymomonas mobilis* genes encoding alcohol dehydrogenase and pyruvate **decarboxylase**. Expression of the genes within the transformant allows the bacterium to produce ethanol as a fermentation product.

L4 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2002 ACS

DUPLICATE 2

ACCESSION NUMBER: 1996:103844 CAPLUS
 DOCUMENT NUMBER: 124:143770
 TITLE: **Ethanol production in**
 Gram-positive microbes
 INVENTOR(S): Ingram, Lonnie O'Neal; Barbosa-Alleyne, Maria D. F.
 PATENT ASSIGNEE(S): University of Florida, USA
 SOURCE: U.S., 11 pp. Cont.-in-part of U.S. Ser. No. 26,051.
 CODEN: USXXAM
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 10
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5482846	A	19960109	US 1994-220072	19940330
US 5000000	A	19910319	US 1989-352062	19890515
US 5424202	A	19950613	US 1992-846344	19920306
CN 1070424	A	19930331	CN 1992-101877	19920318
CN 1065915	B	20010516		
US 5487989	A	19960130	US 1992-946290	19920917
WO 9527064	A1	19951012	WO 1995-US4012	19950330
W: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TT, UA, UZ, VN				
RW: KE, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9522034	A1	19951023	AU 1995-22034	19950330
US 5916787	A	19990629	US 1995-475925	19950607
AU 9918586	A1	19990909	AU 1999-18586	19990305
CN 1342773	A	20020403	CN 2000-131779	20001020
PRIORITY APPLN. INFO.:				
US 1988-239099 B2 19880831				
US 1989-352062 A2 19890515				
US 1990-624227 B2 19901207				
US 1991-670821 B2 19910318				
US 1992-846344 A2 19920306				
US 1992-946290 A2 19920917				
US 1993-260517 A2 19930305				
US 1990-624277 B2 19901207				
US 1993-26051 A2 19930305				
US 1994-220072 A 19940330				
WO 1995-US4012 W 19950330				
AU 1996-61946 A3 19960808				

AB The subject invention concerns the transformation of Gram-pos. bacteria with heterologous genes which confer upon these microbes the ability to produce EtOH as a fermn. product. Specifically exemplified is the transformation of bacteria with genes, obtainable from *Zymomonas mobilis*, which encode pyruvate **decarboxylase** and alc. dehydrogenase.

L4 ANSWER 10 OF 15 BIOTECHDS COPYRIGHT 2002 THOMSON DERWENT AND ISI

ACCESSION NUMBER: 1996-13636 BIOTECHDS

TITLE: Production of recombinant bacterial cellulases by
 ethanologenic bacteria: evaluation for cellulose
 fermentation

; cellulase expression in *Escherichia coli* for improved
 ethanol production (conference abstract)

AUTHOR: Wood B E; Ingram L O

CORPORATE SOURCE: Univ. Florida

LOCATION: University of Florida, Gainesville, FL 32611, USA.

SOURCE: Abstr.Gen.Meet.Am.Soc.Microbiol.; (1996) 96 Meet., 566

CODEN: 0005P

ISSN: 0067-2777

American Society for Microbiology, 96th General Meeting, New Orleans, LA, 19-23 May, 1996.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Previously, *Escherichia coli* KO11 was engineered for fermentation of mixtures of pentose and hexose sugars, and *Klebsiella oxytoca* P2 was engineered for fermentation of cellobiose (from cellulose) to ethanol by integrating the *Zymomonas mobilis* genes for pyruvate-decarboxylase (pdc, EC-4.1.1.1) and alcohol-dehydrogenase (adh, EC-1.1.1.1). In this study, production of recombinant cellulase (EC-3.2.1.4) in KO11 was evaluated during pentose fermentation as a source of supplemental enzymes for cellulose fermentations. Cellulase genes from *Cellulomonas fimi*, *Clostridium thermocellum*, *Erwinia* sp. and *Bacillus subtilis* were tested. In some cases, high levels of cellulase were produced without compromising the ability of KO11 to produce ethanol. Results indicated that it was possible to make significant reductions in the requirement for fungal enzymes by this approach, and show the potential for manufacturing recombinant protein products with ethanol. (0 ref)

L4 ANSWER 11 OF 15 CAPLUS COPYRIGHT 2002 ACS

DUPLICATE 3

ACCESSION NUMBER: 1995:992753 CAPLUS

DOCUMENT NUMBER: 124:28129

TITLE: **Ethanol production with recombinant Gram-positive microbes expressing exogenous pyruvate decarboxylase and alcohol dehydrogenase genes**

INVENTOR(S): Ingram, Lonnie O'Neal; Barbosa-Alleyne, Maria de F.

S.

PATENT ASSIGNEE(S): University of Florida, USA

SOURCE: PCT Int. Appl., 33 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 10

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9527064	A1	19951012	WO 1995-US4012	19950330
W: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TT, UA, UZ, VN				
RW: KE, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 5482846	A	19960109	US 1994-220072	19940330
AU 9522034	A1	19951023	AU 1995-22034	19950330
PRIORITY APPLN. INFO.:			US 1994-220072	A 19940330
			US 1988-239099	B2 19880831
			US 1989-352062	A2 19890515
			US 1990-624227	B2 19901207
			US 1991-670821	B2 19910318
			US 1992-846344	A2 19920306
			US 1992-946290	A2 19920917
			US 1993-260517	A2 19930305
			WO 1995-US4012	W 19950330

AB The subject invention concerns the transformation of Gram-pos. bacteria with heterologous genes which confer upon these microbes the ability to produce ethanol as a fermn. product. Specifically exemplified is the transformation of bacteria with genes, obtainable from *Zymomonas mobilis*, which encode pyruvate decarboxylase and alc. dehydrogenase. A recombinant *Bacillus subtilis* expressing *Z. mobilis* pdc and adhB genes was created.

L4 ANSWER 12 OF 15 CAPLUS COPYRIGHT 2002 ACS

DUPLICATE 4

ACCESSION NUMBER: 1995:5540 CAPLUS

DOCUMENT NUMBER: 122:24802

TITLE: Expression of the *Zymomonas mobilis* alcohol dehydrogenase II (adhB) and pyruvate decarboxylase (pdc) genes in *Bacillus*
AUTHOR(S): Barbosa, Maria de F. S.; Ingram, L. O.
CORPORATE SOURCE: Dep. Microbiol. Cell Sci., Univ. Florida,
Gainesville, FL, USA
SOURCE: Current Microbiology (1994), 28(5), 279-82
CODEN: CUMIDD; ISSN: 0343-8651
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The genes encoding *Zymomonas mobilis* pyruvate decarboxylase (pdc) and alc. dehydrogenase II (adhB) were expressed in *Bacillus subtilis* YB886(pLOI500) under the control of a *Bacillus* SPO2 phage promoter and caused a 50% redn. of growth rate compared with the unmodified vector. Expression was further confirmed by Western blots, activity stains of native gels, and in vitro measurements of alc. dehydrogenase activity. Addnl. strains of *Bacillus* were also transformed, and all produced similar but low levels of these enzymes. Although higher specific activities will be required for efficient ethanol prodn., no fundamental barriers exist to the expression of these *Z. mobilis* genes in *Bacillus*. Two abundant new proteins (ca. mass 33,000 daltons and 14,000 daltons) were obsd. in Coomassie Blue-stained gels; they are similar in size to the proteins induced by recombinant products in *Escherichia coli*.

L4 ANSWER 13 OF 15 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 94:535177 SCISEARCH
THE GENUINE ARTICLE: PD286
TITLE: CONSTRUCTION OF RECOMBINANT PLASMIDS FOR EFFICIENT EXPRESSION OF THE PYRUVATE DECARBOXYLASE GENE (PDK) FROM ZYMMONAS-MOBILIS IN BACILLUS -SUBTILIS
AUTHOR: DANILEVICH V N (Reprint); DUZHII D E; BRAGA E A
CORPORATE SOURCE: MOSCOW GENET & SELECT IND MICROORGANISMS INST, MOSCOW 113545, RUSSIA (Reprint)
COUNTRY OF AUTHOR: RUSSIA
SOURCE: MOLECULAR BIOLOGY, (JAN/FEB 1994) Vol. 28, No. 1, Part 2, pp. 105-110.
ISSN: 0026-8933.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 28

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The pdk gene from *Zymomonas mobilis* localized in a 4.7-kbp SphI fragment of plasmid pB201 was subcloned into the SmaI site of the M13mp19 vector using the DraI restriction endonuclease. The M13mp19 derivatives obtained, carrying a 1.8-kbp DraI fragment in opposite orientations, were used to sequence the pdk gene beginning and end (about 250 bp each) and for site-directed mutagenesis. Using polymerase chain reaction with synthetic oligonucleotide primers, a BamHI site was created in front of the pdk gene initiating codon. The BamHI fragment harboring the pdk gene was cloned into shuttle vector pCB20 under the control of ''expression unit'' EU19035 containing bacillar vegetative promoter and ribosome-binding site (RBS). The pdk gene expression was studied in the recombinant plasmid pCB20pdkI, a derivative of pCB20, which was shown to yield a high level of pyruvate decarboxylase [EC 4.1.1.1] synthesis in *Bacillus subtilis*. However, this plasmid strongly inhibited the *Escherichia coli* cell growth and was eliminated from the cells at a high frequency.

L4 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1992:649986 CAPLUS
DOCUMENT NUMBER: 117:249986
TITLE: Ethanol production by bacteria

carrying foreign genes for alcohol dehydrogenase and
pyruvate decarboxylase

INVENTOR(S) :

Ingram, Lonnie O.; Beall, David S.; Burchhardt,
Gerhard F. H.; Guimaraes, Walter V.; Ohta, Kazuyoshi;
Wood, Brent E.; Shanmugam, Keelnatham T.; Fowler,
David A.; Ben-Bassat, Arie

PATENT ASSIGNEE(S) :

University of Florida, USA; Bioenergy International,
L.C.

SOURCE:

PCT Int. Appl., 153 pp.

CODEN: PIXXD2

DOCUMENT TYPE:

Patent

LANGUAGE:

English

FAMILY ACC. NUM. COUNT:

10

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9216615	A1	19921001	WO 1992-US1807	19920318
W: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE, US RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GN, GR, IT, LU, MC, ML, MR, NL, SE, SN, TD, TG				
US 5424202	A	19950613	US 1992-846344	19920306
AU 9217794	A1	19921021	AU 1992-17794	19920318
AU 672748	B2	19961017		
CN 1070424	A	19930331	CN 1992-101877	19920318
CN 1065915	B	20010516		
EP 576621	A1	19940105	EP 1992-910933	19920318
EP 576621	B1	20010228		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, MC, NL, SE BR 9205782	A	19940726	BR 1992-5782	19920318
AT 199389	E	20010315	AT 1992-910933	19920318
NO 9303178	A	19931108	NO 1993-3178	19930907
CN 1342773	A	20020403	CN 2000-131779	20001020
PRIORITY APPLN. INFO.:			US 1991-670821	A 19910318
			US 1992-846344	A 19920306
			US 1988-239099	B2 19880831
			US 1989-352062	A2 19890515
			US 1990-624277	B2 19901207
			WO 1992-US1807	A 19920318

AB Bacterial hosts, excluding *Escherichia coli*, expressing heterologous genes

for alc. dehydrogenase (I) and pyruvate decarboxylase (II) are used for manuf. of EtOH. II is used to prevent accumulation of acid metabolites. Plasmids, e.g. pLOI555 carrying genes for I and II of *Zymomonas mobilis* driven by the lac promoter, are provided for prepn. of the host. The method is further improved by transforming the host with genes for proteins that facilitate transport and metab. of oligosaccharides, e.g., of C5-6 sugars, which host is, preferably, also expressing a heterologous gene for a polysaccharase such as a cellulolytic

enzyme, a xylanolytic enzyme, or a starch-degrading enzyme. These hosts also preferably express heterologous genes for polysaccharide- degrading enzymes (e.g. those degrading cellulose, xylans, or starch). A cost-effective fermn. process for manufg. EtOH from oligosaccharide feedstocks using a single, genetically engineered microorganism is also disclosed. An ethanologenic strain *Klebsiella oxytoca* M5A1(pLOI555) was prep'd. and was further transformed with plasmid pLOI2003 encoding xylanase

(gene *xynZ*) and xylosidase (gene *xylB*) of *Clostridium thermocellum* to obtain a transformant capable of converting xylan to EtOH.

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ULRICH BUSCH I; SAHM H; SPRENGER G A (preprint)
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ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB An approach to broaden the product range of the ethanologenic, gram-negative bacterium *Zymomonas mobilis* by means of genetic engineering is presented. Gene *alaD* for L-alanine dehydrogenase (EC 1.4.1.1) from *Bacillus sphaericus* was cloned and introduced into *Z. mobilis*. Under the control of the strong promoter of the pyruvate **decarboxylase** (*pdc*) gene, the enzyme was expressed up to a specific activity of nearly 1-mu-mol . min-1 . mg of protein-1 in recombinant cells. As a result of this high L-alanine dehydrogenase activity, growing cells excreted up to 10 mmol of alanine per 280 mmol of glucose utilized into a mineral salts medium. By the addition of 85 mM NH4+ to the medium, growth of the recombinant cells stopped, and up to 41 mmol of alanine was secreted. As alanine dehydrogenase competed with pyruvate **decarboxylase** (PDC) (EC 4.1.1.1) for the same substrate (pyruvate), PDC activity was reduced by starvation for the essential PDC cofactor thiamine PP(i). A thiamine auxotrophy mutant of *Z. mobilis* which carried the *alaD* gene was starved for 40 h in glucose-supplemented mineral salts medium and then shifted to mineral salts medium with 85 mM NH4+ and 280 mmol of glucose. The recombinants excreted up to 84 mmol of alanine (7.5 g/liter) over 25 h. Alanine excretion proceeded at an initial velocity of 238 nmol . min-1 . mg [dry weight]-. Despite this high activity, the excretion rate seemed to be a limiting factor, as the intracellular concentration of alanine was as high as 260 mM at the beginning of the excretion phase and decreased to 80 to 90 mM over 24 h.